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Genetic diversity of milk protein beta-lactoglobulin and association with production traits genomic values among Holstein cattle

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ABSTRACT

The aim of the study was to evaluate the prevalence of beta-lactoglobulin genotypes in Lithuanian Holstein dairy cattle population and to identify possible synergies between the different genotypes of beta-lactoglobulin and genomically predicted values for milk production traits. DNA samples were collected from Holstein cattle (147) and bovine beta-lactoglobulin gene polymorphism study was performed by PCR-RFLP method. A allele was identified with frequency 0.456, B allele, which can be used to carry out selection to improve milk processing properties, was found with 0.544 frequency. Three genotypes, viz. AA, AB, BB at different frequencies were identified. The biggest influence on the milk processing properties having BB genotype was found in 27.3% of the cows. Beta-lactoglobulin AA genotype cows had higher average genomic values for milk yield, while BB genotype cows had higher average genomic values for milk protein percentage, the differences have found statistically significant. Dispersion analysis showed that beta-lactoglobulin genotype influences 8.4% of milk amount genetic variation, 1.2% of milk protein amount genetic variation and 20.6% of milk protein percent genetic variation. Lithuanian Holstein cows population had higher average genomic prediction values for milk yield, milk protein amount and milk percentage than average genomic values of Igenity reference animal group. The existence of the most important genotype BB of beta-lactoglobulin for milk manufacturing properties, in studied population increases the possibility of selecting cows according to milk protein polymorphism, and could be economically important selection criteria for dairy herds designated for industrial milk production.

Key words: Beta-lactoglobulin, Genomic selection, Holstein cattle, Polymorphism

Genetic variants (A and B) found in beta-lactoglobulin locus may be associated with lactation process and have a lot of influence on milk composition. Caseins are proteins that are clotted during cheese manufacturing process, consequently the higher casein content the better the cheese yield from the same quantity of milk is obtained (Anggraeni *et al.* 2017). Studies have found that BB genotype determines 3% more amount of casein, than AA genotype, so the BB genotype cows with the same volume of milk can produce about 2% more cheese. For these reasons, milk from cows with BB genotype is more desirable in cheese production (Ganai *et al.* 2012, Petrovska *et al.* 2017).

It is known that the distribution of alleles can vary quite strongly in the different breeds of cattle (Gouda *et al.* 2011, Doostl *et al.* 2011, Lukac *et al.* 2013, Gedik *et al.* 2016). Nowadays the increase of desired allele frequency in the population and the ability to choose valuable beta-lactoglobulin genotype is a very important goal for breeders. Therefore, our aim was to evaluate the prevalence of beta-lactoglobulin genotypes in Holstein dairy cattle population in Lithuania, including the identification of possible synergies between the different genotypes of beta-lactoglobulin and genomically predicted milk production traits.

MATERIALS AND METHODS

Blood sample collection: Holstein cows (147), reared in Lithuania, were examined in this study. Blood samples were collected by puncture of jugular vein into sterile EDTA containing tubes, transported to laboratory and stored at –20°C until genomic DNA extraction. Data of milk yield, milk protein amount, milk protein percent were received from Milk recording center.

DNA extraction: DNA was extracted from white blood cells (leukocytes) by chloroform salt method. Genomic DNA content and quality were determined by spectro-photometric method (DNA / RNA Reader, Pharmacia). Genomic DNA was stored at 4°C until analysis.

Polymerase chain reaction: Genotyping for beta-

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Table 1. Primers, PCR profile, PCR product size and restriction enzymes used for identification of milk protein beta-lactoglobulin polymorphism

Milk protein	Primers	PCR profile			PCR product size	Restriction enzyme
Beta-lactoglobulin	JBLG 2: 5'- TGT GCT CAC CGA GGA CTA CAA AAA G-3'	94°C	3 min		247 bp	HaeIII
		94°C	40 sec			
	JBLG 3: 5' - GCT CCC GGT ATA TGA CCA CCC TCT-3'	58°C	50 sec	35 cycles		
		72°C	50 sec			
		73°C	5 min			

Table 2. PCR fragments sizes in bp of beta-lactoglobulin gene after the digestion with restriction endonuclease

Genotype	Fragment sizes in bp
A/A	148 + 99
A/B	74+74+99+99+148
B/B	74 + 74 + 99

lactoglobulin was done using PCR-RFLP method (Peèiulaitiene *et al.* 2007) (Table 1).

Restriction fragment length polymorphism (RFLP) technique: A single nucleotide polymorphisms of the bovine beta-lactoglobulin gene located on chromosome 11 based on the restriction fragment length polymorphism were detected. PCR product was digested with 10 µl restriction mix (7.5 µl ddH_2O, 2 µl 10× Mbuf., 0.5 µl. *HaeIII*). The samples were left in the thermostate for night (15 h) at 37°C. Restricted PCR product was fractionated by electrophoresis method on 4% agarose gel, 100 V for 50 min. Gel was stained with ethidhium bromide for 15–20 min and restricted DNA bands were analysed under UV light (wave length 300 nm) by video documentation system. Size of different DNA bands resulting from restriction with enzyme was confirmed through standard DNA marker and the size of unrestricted PCR product was taken as control. After digestion of 247 bp PCR products with restriction enzyme *HaeIII* 3 genotypes were determined (Table 2).

Genomic prediction values for production traits: Genomic prediction values for milk yield, protein amount, protein percentage were determined by Igenity SNP panel identifying the genetic potencial of animals for certain traits. The Igenity dairy cattle profile shows genetic prediction values by genomic scores from 1 to 10 for traits using multiple DNA SNP markers. The largest score indicates the best genomic value and the largest genetic potencial of the animal for certain trait. The profile calculates scores for milk yield, protein amount and protein % using multiple DNA markers. These markers identify genetic variations, that help regulate milk yield and protein content, without decreasing fertility. Combined results provide more complete picture of an animal's production genetic potential. The tested animals have been rated in Igenity reference group (http://genomics.neogen.com/en/).

Statistical analysis performed by SPSS program 20.0 for Windows. The alleles and genotypes frequencies of beta-lactoglobulin polymorphism were examined for deviation from Hardy-Weinberg equilibrium using X^2 test. The effect of beta-lactoglobulin genotype on milk production traits in standard length of lactation - milk, protein yield and protein, % was determined with the One-Way ANOVA procedure (post-hoc Fisher LSD criterion (α=0.05)).

Experiments performed comply with national and EU current laws (National Animal Care Authority law, http://conventions.coe.int/treaty/en/treaties/html/123.htm).

RESULTS AND DISCUSSION

The frequencies of beta-lactoglobulin genotypes and alleles found in the studied population of Holstein cows are presented in Table 3. In current study, beta-lactoglobulin genotype distribution for the tested Holstein population indicates Hardy-Weinberg genetic equilibrium (P=0.50). BB genotype, which highly influences the processing properties of milk, was found in 27.3% of Lithuanian Holstein cows population.

Beta-lactoglobulin AA genotype cows had increased milk yield per lactation, BB genotype cows had increased protein percentage, though differences were not statistically reliable (Table 4).

The highest predicted genomic values for milk yield had beta-lactoglobulin AA genotype cows, 53% of cows with AA genotype had genomic scores higher than 6, while only 16.1% of beta-lactoglobulin BB genotype cows had genomic scores higher than 6. The highest genomic score for the whole tested population was 9. 6.1% of cows had

Table 3. Alleles and genotypes frequencies of beta-lactoglobulin in Lithuanian Holstein cattle

Cows	Genotype			Allele		X^2
	AA	AB	BB	A	B	1.38
Number	27	80	40	134	160	
Frequency	0.183	0.544	0.273	0.456	0.544	

Table 4. Means and standard deviations of milk production traits in cows with different beta-lactoglobulin genotypes

Genotype	n	Traits (means±SD)		
		Milk yield, kg	Protein, %	Protein, kg
AA	27	8472.42±1802.80	3.22±0.36	293.28±40.7
AB	80	8120.69±1885.55	3.21±0.32	288.58±56.46
BB	40	8346.72±1636.39	3.32±0.33	235.5±42.37

very high predicted value for milk yield showing high genetic potential and good opportunities for selection. The influence of different genotypes of beta-lactoglobulin to the amount of milk is statistically significant (P<0.01) receiving a higher percentage of the higher scores in AA genotype (Table 5).

The highest predicted genomic values for milk protein amount had beta-lactoglobulin AA genotype cows, 42.0% of cows with AA genotype had genomic scores higher than 6, while 16.1% of beta-lactoglobulin BB genotype cows had genomic scores higher than 6. The influence of different genotypes of beta-lactoglobulin to the amount of protein were not statistically significant (P>0.01) with tendency to receive a higher percentage of higher scores in AA genotype (Table 6).

The highest predicted genomic values for milk protein percentage had beta-lactoglobulin BB genotype cows, 32.3% of cows with BB genotype had genomic scores higher than 6, while genomic scores higher than 6, had only 16.0% of AA genotype cows, 9.7% of beta-lactoglobulin BB genotype cows had the highest genomic score 10, for protein percentage, showing great possibilities for selection according to desirable trait. The influence of different genotypes of beta-lactoglobulin to the milk protein percentage was statistically significant (P<0.001) receiving a higher percentage of the higher scores in BB genotype (Table 7).

Beta-lactoglobulin AA genotype cows have higher average genomic score for milk yield, while BB genotype cows have higher average genomic score for milk protein percentage, differences were statistically significant (P<0.001) (Table 8).

Factorial dispersion analysis showed that beta-lactoglobulin genotype influenced 8.4% of milk amount genetic variation (P<0.01), 1.2% of milk protein amount genetic variation, and 20.6% of milk protein percent genetic variation (P<0.001).

Cows from tested Lithuanian Holstein population had higher average genomic prediction values for milk yield, milk protein amount and protein percentage than average genomic values of Igenity reference animal group (Table 9).

Relationships between milk protein polymorphism, production traits, composition of milk and milk manufacturing properties and effect of the different proteins loci on quantitative traits have been studied and described in several studies (Michalcova *et al.* 2007, Petrovska *et al.* 2017). There are 12 alleles identified of beta-lactoglobulin, but the A and B alleles are the most prominent (Tetens *et al.* 2014, Jebin *et al.* 2016). Beta-lactoglobulin C alelle is not often found and only in several breeds - Australian Jersey breed, Siboney de Cuba hybrids, German Jersey, Polish Red Cattle and German Red Cattle, Lithuanian Red (Miceikienë *et al.* 2006). Beta-lactoglobulin D allele was found in Polish Simental breed (Felenczak *et al.* 2008). Other beta-lactoglobulin variants are very rear and usually found only in one breed.

Beta-lactoglobulin A and B variants are diffused in many dairy cattle breeds. B variant is the most common to many cattle breeds. In Brown Swiss BB genotype has been found

Table 5. Distribution of cows with different beta-lactoglobulin genotypes according to predicted milk yield genomic values

Beta-lactoglobulin genotypes	Percentage of cows' milk yield genomic scores from 1 to 10									
	1	2	3	4	5	6	7	8	9	10
AA			2.0%	10.2%	10.2%	24.5%	24.5%	22.4%	6.1%	
AB		3.2%	6.5%	10.8%	22.6%	29.0%	20.4%	5.4%	2.2%	
BB			3.2%	25.8%	12.9%	41.9%	16.1%			

Table 6. Distribution of cows with different beta-lactoglobulin genotypes according to predicted protein amount of genomic values

Beta-lactoglobulin genotypes	Percentage of cows with protein amount (kg) genomic scores from 1 to 10									
	1	2	3	4	5	6	7	8	9	10
AA		6.0%	6.0%	8.0%	18.0%	20.0%	20.0%	16.0%	4.0%	2.0%
AB	1.1%	5.4%	2.2%	17.2%	15.1%	16.1%	15.1%	16.1%	9.7%	2.2%
BB			3.2%	12.9%	3.2%	32.3%	16.1%	22.6%	6.5%	3.2%

Table 7. Distribution of cows with different beta-lactoglobulin genotypes according to predicted protein percentage genomic values

Beta-lactoglobulin genotypes	Percentage of cows with protein (%) genomic scores from 1 to 10									
	1	2	3	4	5	6	7	8	9	10
AA					16.0%	40.0%	28.0%	10.0%	6.0%	
AB					1.1%	14.0%	31.2%	31.2%	18.3%	4.3%
BB					3.2%	6.5%	25.8%	32.3%	22.6%	9.7%

Table 8. Mean genomic scores for production traits in cows with different beta-lactoglobulin genotypes

Beta-lactoglobulin	n	Average genomic scores		
		Milk (kg)	Protein (kg)	Protein (%)
AA	27	6.51±0.211[b]	5.96±0.268	6.50±0.152[b]
AB	80	5.61±0.155[a]	6.02±0.213	7.65±0.114[a]
BB	40	5.42±0.206[a]	6.55±0.300	7.94±0.217[a]

Table 9. Comparison of average genomic values of dairy cattle in Lithuania with Igenity reference group animals

Traits	Average genomic values of tested dairy animals	Average genomic values of Igenity reference animal group
Milk, kg	6.48	5.93
Protein, kg	6.18	6.36
Protein, %	7.36	6.67

in 28.97% of cattle, in Ayrshire breed BB genotype had 49%, in Jersey breed 45%, in Egyptian Baladi breed 74% of tested cattle (Gouda *et al.* 2011). In Iranian native beta-lactoglobulin B allele was found in frequency of 0.77 (Doostl *et al.* 2011). In Czech Fleckvich cattle beta-lactoglobulin B allele was found in frequency of 0.489 (Kucerova *et al.* 2006).

In the present study we have investigated beta-lactoglobulin polymorphism in Lithuanian Holstein cattle. We have found 27% of Lithuanian Holstein cows with beta-lactoglobulin BB genotype, 18% with AA genotype, 54% with AB genotype. Our findings coincident with other studies - in Croatian Holstein BB genotype was found in 24% of cows, in Serbian Holstein in 19% (Lukac *et al.* 2013), in Turkey varying from 15 to 39% (Gedik *et al.* 2016).

Most favourable for manufacturing milk properties B allele was found with frequency 0.54 in Lithuanian Holstein cattle. Relatively high frequency of beta-lactoglobulin B allele has been found in Holstein cattle breed in Turkey (0.49–0.66) (Dinc *et al.* 2013, Gedik *et al.* 2016, Zaglool *et al.* 2016) in Iran (0.47) (Doostl *et al.* 2011), in Serbia (0.48) (Lukac *et al.* 2013, Vidovic *et al.* 2014), slightly lower in Polish Holstein breed (0.34), in Thailand (0.29), high in Egyptian Holstein (0.87) (Gouda *et al.* 2011), in Chinese Holstein (Ren *et al.* 2011, Alim *et al.* 2015), in Estonia (0.68) (Varv *et al.* 2009).

Several studies have confirmed that beta-lactoglobulin polymorphism influences not only manufactoring properties of milk, but also production traits such as milk yield, milk protein percentage and milk protein amount (Zaglool *et al.* 2016).

For the first time we reported the associations of Igenity dairy cattle genomic prediction values for production traits with milk protein genotypes. Cows with beta-lactoglobulin AA genotype had the highest genomics values for milk yield, beta-lactoglobulin BB genotype cows had the highest genomic values for protein amount and protein percentage. We have received the same tendencies of beta-lactoglobulin polymorphism influence to production traits expressed in kg for milk yield, percentage for milk proteins and kg for milk protein amount as well as by genomic value prediction scores (varying from 1 to 10). So having high genomic value scores in milk yield we can predict prevailing AA beta-lactoglobulin genotype in tested herd, while having high genomic value scores in milk protein percentage we can predict prevailing BB beta-lactoglobulin genotype in tested herd.

The existence of being the most important for milk manufacturing properties genotype BB of beta-lactoglobulin in studied population increases the possibility of selecting cows according to milk protein polymorphism and could be an economically important selection criteria for dairy herds, designated for industrial milk production.

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